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The Role of Vascular Endothelial Growth Factor in Systemic Sclerosis

Abstract

The pathological hallmarks of Systemic sclerosis (SSc) constitute an inter-related triad of autoimmunity, vasculopathy and tissue remodeling. Many signaling mediators have been implicated in SSc pathology; most focusing on individual components of this pathogenic triad and current treatment paradigms tend to approach management of such as distinct entities. The present review shall examine the role of vascular endothelial growth factor (VEGF) in SSc pathogenesis. We shall outline potential mechanisms whereby differential vascular endothelial growth factor-A (VEGF-A) isoform expression (through conventional and alternative VEGF-A splicing,) may influence the relevant burden of vasculopathy and fibrosis offering novel insight into clinical heterogeneity and disease progression in SSc. Emerging therapeutic approaches targeting VEGF signaling pathways might play an important role in the management of SSc, and differential VEGF-A splice isoform expression may provide a tool for personalized medicine approaches to disease management.

Key words

Systemic sclerosis (scleroderma)
Pathogenesis
Vascular endothelial growth factor
VEGF-A
VEGF-A_{165}b
Fibrosis
Vasculopathy
Anti-angiogenic
Pro-fibrotic

Abbreviations:

ACA anti-centromere autoantibody, Ang angiopoietin, BM basement membrane, Cav caveolin, CCH, chronic continuous hypoxia, CIH chronic intermittent hypoxia, CTGF connective tissue growth factor, dcSSc diffuse cutaneous SSc, DU digital ulceration, ECM extracellular matrix, EndoMT endothelial-to-mesenchymal transition, ERA endothelin-1 receptor antagonists, HIF hypoxia inducible factor, HRE hypoxia response elements, HSP heparin sulphate proteoglycan, HSC haemopoietic stem cells, Id1 Inhibitor of DNA binding protein-1, IL-1β Interleukin 1β, IL6 interleukin-6, ILD interstitial lung disease, IPF idiopathic pulmonary fibrosis, lcSSc limited cutaneous SSc, mRSS modified Rodnan skin score, MSC mesenchymal stem cell, MVEC microvascular endothelial cell, NC nailfold capillaroscopy, NO nitric oxide, NP neuropilin, PAH pulmonary arterial hypertension, PDE5 phosphodiestrase-5, PDGF platelet derived growth factor, PDGFR platelet derived growth factor receptor, PlGF placental growth factor, RNAPIII anti-RNA polymerase III autoantibody, RP Raynaud's phenomenon, Scl-70 anti-topoisomerase autoantibody, SSc Systemic sclerosis, TK tyrosine kinase, TGFβ transforming growth factor-β , TNFα tumour necrosis factor-α , VEGF vascular endothelial growth factor, VEGFR vascular endothelial growth factor receptor.

Introduction

Systemic sclerosis (SSc) is a rare multisystem autoimmune disease whose pathological hallmarks constitute a triad of vasculopathy, autoimmunity and aberrant tissue remodeling, manifesting as Raynaud's phenomenon (RP), circulating autoantibodies and cutaneous fibrosis (scleroderma) respectively. SSc is a heterogeneous disease whose clinical phenotype and major subgroup classifications are largely defined by the relative presence and extent of tissue fibrosis and vasculopathy. Autoantibody expression has strong associations with particular clinical phenotypes[1] but these associations are not absolute and the presence and severity of clinical features vary widely between individuals. Despite this, management approaches are relatively uniform focusing predominantly on immunosuppression and vasodilation[2]. LeRoy was the first to propose an important inter-relationship between the pathological hallmarks of the disease, suggesting vasculopathy as an important driver of tissue remodeling and autoimmunity[3]. Many signaling pathways have been implicated in SSc pathogenesis with a significant focus on the pro-fibrotic potential of transforming growth factor-β (TGFβ), platelet derived growth factor (PDGF), connective tissue growth factor (CTGF), serotonin and interleukins[4], although therapeutic trials targeting specific pro-fibrotic molecular targets to date have been disappointing[5-7].

Therapeutic approaches targeting the molecular pathways relating to vasculopathy and tissue hypoxia have been less extensively studied in SSc but emerging evidence suggests an important role of proteins including hypoxia inducible factor (HIF)[8] and vascular endothelial growth factor (VEGF)[9-14] in SSc pathogenesis. Indeed, recent clinical trials demonstrating the efficacy of small molecule tyrosine kinase inhibitors targeting VEGF receptor signaling[15] has led to renewed interest in the potential role of VEGF in SSc. This review examines the available evidence of VEGF signaling in SSc and explores the potential contribution to SSc pathogenesis. We shall describe emerging evidence concerning the competing influence of differential VEGF splice isoform expression and the potential implications for therapeutic approaches targeting VEGF in SSc.

Vasculopathy in Systemic sclerosis

Endothelial injury is an important initiating event in SSc and vasculopathy occurs early in the disease process. Endothelial dysfunction and apoptosis, increased vascular permeability, vessel wall remodeling, platelet aggregation, and a perivascular inflammatory cell infiltrate pre-date the development of established tissue fibrosis[16-18]. Clinical manifestations of RP and morphological capillary changes at the nailfold (Figure 1) precede the onset of overt cutaneous fibrosis by an average of four years[19, 20]. A 20-year prospective study following the disease course of patients with RP, noted transition on nailfold capillaroscopy (NC) from giant capillaries to capillary loss occurring in close temporal relationship to the emergence of clinical features that led to a diagnosis of definite SSc (usually defined by the emergence of cutaneous fibrosis)[20]. A number of studies have also identified a positive and progressive association between the severity of microangiopathy on NC and the extent of skin fibrosis[21-25]. Indeed, 'early' changes are more frequently identified in limited cutaneous SSc (lcSSc)[23, 26] whereas 'late' changes are more prevalent in diffuse cutaneous SSc (dcSSc)[19, 25]. The rate of progression of NC changes (and skin fibrosis) varies according to autoantibody specificities. For example, SSc patients carrying anti-RNA polymerase III autoantibodies (RNAPIII) have been noted to develop enlarged capillaries and capillary loss earlier in the disease course (4 vs. 15 years) than patients with anti-centromere autoantibody (ACA)[20].

Unsurprisingly, the progression of NC changes, predict the severity of peripheral vascular manifestations. For example, in lcSSc and dcSSc, capillary loss that accompanies 'late' NC pattern carries an increased risk of developing new digital ulcers (DU) compared with preserved capillary number seen in 'normal' or 'early' patterns[23, 27]. In that vein, DU are more common in dcSSc than lcSSc[28]. However, whilst DU are significantly less common in SSc sine scleroderma (ssSSc), no association with NC pattern has been identified in this subset, perhaps reflecting low study numbers[28]. Larger capillary loop diameter and greater numbers of giant capillaries have also been used to predict the future development of DU in SSc, demonstrating the association between disorganized neoangiogenesis and inadequate tissue perfusion[29].

The principle pathological effect of vasculopathy in SSc is tissue hypoxia. Indeed, oxygen saturations in fibrotic skin of SSc patients are notably low (pO_2 23.7+/-2.1mmHg compared to 33.6+/-4.1mmHg in healthy controls ($p<0.05$))[11]. Tissue hypoxia in SSc is further exacerbated by the oxygen demands of

the inflammatory milieu and impaired oxygen diffusion secondary to aberrant tissue remodeling and accumulation of extracellular matrix proteins.

Tissue hypoxia leads to over-expression of cell signaling molecules such as HIF and VEGF that protect cells from oxidative stress, promote wound healing and enhance tissue perfusion through neoangiogenesis[30].

Hypoxia inducible factor (HIF)

HIF is a family of transcription factors within the PER ARNT SIM transcription group[31]. HIF is a heterodimer consisting of an α subunit (HIF1α, 2α, 3α; whose expression is dependent on local tissue oxygenation[32]) and a β subunit (a constitutively expressed nuclear protein)[31]. Under normoxic conditions, HIFα is rapidly degraded by proteasomes and is therefore only detected at significant levels under hypoxic conditions[31]. As HIFα accumulates, the HIFα/β dimer (hereafter referred to as HIF1, 2, 3) binds to hypoxia response elements (HRE) to up-regulate gene transcription[32]. HIF1α is expressed widely throughout almost all tissues, whereas HIF2α paralog is differentially expressed in endothelium, renal, hepatic, pulmonary and brain tissue[33]. HIF1 has been implicated in a number of diseases characterized by altered angiogenesis, inflammation and fibrosis [32, 34] including pulmonary arterial hypertension (PAH)[35], which can occur as a manifestation of SSc related vasculopathy.

Vascular endothelial growth factor (VEGF) family

The VEGF family comprises placental growth factor (PlGF) and four mammalian VEGF subgroups (VEGF A-D). Figure 2 illustrates the biological actions of VEGF receptors (VEGFR) and the associated co-receptors.

VEGF-A was initially described as a vascular permeability factor[36] and subsequently shown to exhibit both mitogenic and angiogenic properties[37, 38]. Levels are precisely controlled such that a single allele deletion results in embryonic failure[39] and VEGFR1 gene mutation causes disorganized endothelial cell lining and failed angiogenesis[40]. However, its biological actions now appear wider, including neutrophil chemo-attraction[41] and fibrosis[42]. Hypoxia is a major up-regulator of VEGF-A both via up-regulation of HIF[32] and via hypoxic VEGF-A mRNA stabilization[43].

Variable splicing of the 6th and 7th exons of VEGF-A results in different isoforms (hereafter referred to as VEGF-A_{xxx}a); named according to their respective number of amino acids (e.g. VEGF-A_{165}a)[44]. VEGF-A_{165}a is the dominant pro-angiogenic factor amongst the VEGF-A family acting through the principle receptor (VEGFR2)[45], to stimulate neoangiogenesis through proliferation and migration of endothelial cells to form new tubular vessel structures[46].

Until recently, all VEGF isoforms were considered pro-angiogenic factors. However, since 2002 a number of alternative splice variants of VEGF-A (VEGF-A_{xxx}b and VEGF-Ax) have been identified, some of which inhibit angiogenesis through competitive binding of VEGFR2[47-49] and absence of neuropilin-1 (NP-1) co-receptor binding (Figure 2)[50]. The latter also directs alternative intracellular trafficking in favour of VEGFR2 degradation[51]. However, VEGF-A_{xxx}b isoforms may have physiologically beneficial roles in placental neoangiogenesis and pre-eclampsia during pregnancy[52] and the inhibition of tumour growth and metastatic progression in many cancers[47].

Other members of the VEGF family (VEGF-B-D and PIGF) also promote angiogenesis through co-binding of VEGFR1 and NP-1[53]. VEGF-B also plays a role in fatty acid transport and may provide a therapeutic target for insulin resistance and type 2 diabetes[53]. VEGF-C and VEGF-D signal through an alternative VEGFR3, promoting lymphangiogenesis in embryonic and postnatal periods [53].

The potential role of VEGF in SSc related vasculopathy

In view of the characteristic microvascular manifestations of SSc, VEGF-A pathways have attracted interest as potentially important drivers of disease pathogenesis. In view of the pronounced capillary drop out found in SSc, the authors of early studies were surprised to identify high levels of circulating serum VEGF-A in both early[9] and established SSc [22, 54], although serum VEGF-A was noted to be comparatively lower in SSc patients with DU[9, 55]. VEGF associations with systemic organ manifestations of SSc have been less extensively studied and are notably varied. Circulating VEGF-A

levels in SSc pulmonary vasculopathy are contradictory[56, 57]. Limited data shows no correlation between elevated serum VEGF-A with ultrasound parameters of renal vasculopathy[58]. One study has reviewed VEGF-A through non-invasive sampling of tears of SSc patients and found levels to be surprisingly low possibly explained by reduced tear secretion associated with dry eye syndrome[59].

The elevated VEGF-A levels initially appeared at odds with the obliterative microangiopathy associated with progressive capillary loss[9, 60, 61]. A proposed explanation for these apparently conflicting findings is cellular compartmentalization of VEGF-A and its receptors; a biological concept that might be important in healthy lung homeostasis[62]. However, the identification of VEGF-A splice variants with opposing angiogenic function provides a deeper and more compelling explanation. VEGF-A_{165}a and VEGF-A_{165}b isoforms differ by only six amino acids at exon 8. Commercially available VEGF-A ELISAs are unable to differentiate between these isoforms. Thus, aforementioned studies[9, 22, 54, 60, 61] likely detected pan-VEGF-A (representing co-detection of VEGF-A_{xxx}a and VEGF-A_{xxx}b soluble isoforms). Subsequent studies used isoform VEGF-A_{165}b specific detection methods to confirm an association between VEGF-A_{165}b and the 'late' avascular patterns on NC[14]. Furthermore, those with 'early' nailfold changes (i.e. few microvascular changes) have similar VEGF-A_{165}b levels to healthy controls[14], suggesting that anti-angiogenic isoform expression evolves with disease progression, although longitudinal studies have yet to confirm this.

VEGF receptor status in SSc skin, serum and cell culture is mixed and inconclusive[11, 63-66]. However, higher levels of circulating soluble VEGFR2 appear to be associated with telangiectasia[64]. Urokinase-type plasminogen activator receptor (uPAR), which is required for VEGFR2 internalization, is reduced in SSc skin[50]. Additionally, NP-1 is reduced in skin and serum[50, 65] and associates with DU and more advanced (active/late) NC patterns in SSc[65]. Interestingly, despite evidence of microvasculopathy at the nailfold, reduced serum NP-1 does not appear to associate with specific NC patterns in those with pre-SSc[67]. However, exposure of MVEC to patient sera attenuates NP-1 expression; a phenomenon demonstrated even by sera from pre-SSc donors[67]. In combination, the functional status of VEGFR2 appears to be impaired from multiple co-factors, potentially reducing the pro-angiogenic potential of VEGF-A_{xxx}a and potentiating VEGF-A_{xxx}b inhibitory action.

Given the relationship between NC pattern and VEGF-A_{165}b, and known correlations between capillary density and both gas transfer[22] and the presence of SSc-related pulmonary disease (both interstitial lung disease (ILD) and PAH)[68], the relationship between inhibitory VEGF-A_{xxx}b isoforms and pulmonary vasculopathy is of interest but has not been investigated to date. Interestingly, transgenic mice over-express anti-angiogenic pulmonary VEGF-A_{165}b do not develop vascular abnormalities[42] whereas overproduction of VEGF-A_{164}a (the murine equivalent of VEGF-A_{165}a) results in increased vessel number and wall thickness[69] and dilated and disorganized vasculature[70] suggesting it is the relative rather than absolute level of the anti-angiogenic isoforms that dictates vascular morphology.

The roles of VEGF-B-D have been less extensively investigated in SSc. VEGF-C/D regulate lymphangiogenesis and lymphatic endothelium (Figure 2). SSc lesional skin displays a progressive reduction in lymphatic number[71, 72] despite the fact that circulating VEGF-C and cutaneous VEGF-D[73] and its receptor (VEGFR3)[72] are increased. This might indicate impaired downstream signaling of VEGFR3 or the presence of splice variants of VEGF-C/D with opposing functions; akin to the aforementioned VEGF-A_{xxx}b isoforms. In one study, plasma VEGF-D levels were shown to increase at the time of PAH diagnosis[74].

The potential role of VEGF-A in fibrosing disease

HIF and VEGF-A have been implicated in a number of fibrosing diseases including graft versus host disease, hepatic fibrosis and idiopathic pulmonary fibrosis (IPF) [75-82].

A pro-fibrotic role for VEGF-A_{165}a in SSc is supported by the demonstration of increased collagen induction in both healthy and SSc dermal fibroblasts in response to VEGF-A_{165}a with more pronounced effects observed in SSc fibroblasts[83]. Serum panVEGF-A levels in SSc correlate with skin scores[60] and increased levels are associated with dcSSc[9, 60] and anti-topoisomerase autoantibodies (anti-Scl-70)[9, 84]. PanVEGF-A and HIF1α are increased in SSc hypoxic lesional skin[8, 11] and over-expressed by dermal fibroblasts cultured under hypoxic conditions[85]. Furthermore, panVEGF-A is overexpressed in non-lesional skin predating the onset of fibrosis[11] implicating VEGF-A as an early signaling protein

in fibrosis. However, whilst lesional skin is notably hypoxic, the pO_2 of non-lesional skin is normal[11] suggesting that VEGF-A expression may be stimulated by factors beyond hypoxia.

To date, the focus of VEGF-A_{165}b investigation in SSc has been with regard to its anti-angiogenic function and there are few observations reported with regard to fibrosis in SSc. Studies have identified increased VEGF-A_{165}b in the skin (mRNA) and plasma of SSc patients[12, 14], which may account for the majority of panVEGF-A overexpression[12]. VEGF-A_{165}b appears to be particularly elevated in certain autoantibody profiles (anti-centromere and anti-Scl-70)[86, 87] although, in contrast to panVEGF-A, VEGF-A_{165}b levels (in skin and plasma) do not apparently correlate with extent of skin involvement[12, 14]. Recent data from murine models of an alternative fibrotic disease (IPF), suggest that VEGF-A_{xxx}a isoforms act as pro-fibrotic drivers of IPF whilst the VEGF-A_{165}b isoform has opposing anti-fibrotic properties[42]. In this model, the balance between VEGF-A_{165}a and VEGF-A_{165}b expression may also be important in IPF pathogenesis[42]. Considering this parallel fibrotic disease, it may be hypothesized that VEGF-A_{165}b could detrimentally contribute to the progressive vasculopathy in SSc whilst encouraging regression of skin fibrosis later in disease. Interestingly, VEGF-A_{165}b appears to be higher in the skin of early SSc (despite comparable circulating plasma levels)[12]. This may suggest the occurrence of early isoform switching.

There is conflicting data regarding the association of circulating panVEGF-A levels with the degree of pulmonary fibrosis on computerized tomography[22, 57, 60, 88]. As discussed for SSc-PAH, VEGF-A_{xxx}b isoform expression has not been specifically studied and conflicting reports in SSc-ILD may be a consequence of panVEGF-A detection.

What is the potential cellular source of VEGF-A isoforms in SSc?

If VEGF-A is at the forefront of disease initiation then identifying its cell origin is paramount to understanding and modifying its signaling network. In SSc, panVEGF-A and VEGF-A_{165}b is expressed in fibroblasts, endothelial and perivascular inflammatory cells[11, 12] with additional expression of VEGF-A_{165}b in vascular smooth muscle cells in *ex vivo* lesional skin[12]. Circulating mononuclear cells[89] and skin keratinocytes[8, 11] also produce increased panVEGF-A levels.

In vitro, cultured microvascular endothelial cells (MVEC) express higher VEGF-A_{165}b (co-localized with increased VEGFR2 but with impaired signaling function) than controls[12] (Figure 3). Additionally, when MVEC, from non-lesional SSc skin, are co-cultured in vitro with activated fibroblasts from lesional skin, panVEGF-A and CD31 expression in the former are reduced whilst VEGF-A_{165}b is increased[90]. This is associated with reduced microtubule formation and increased endothelial-to-mesenchymal transition (EndoMT)[90], demonstrating the potential paracrine activity of SSc fibroblasts on the vasculature and potential to perpetuate the cycle posed by the vascular hypothesis[90].

Platelets are an important source of circulating panVEGF-A in SSc[91] and recent investigation has also proven them to be an important source of VEGF-A_{165}b[92]. Furthermore, tubule formation by dermal MVEC in vitro is impaired when incubated with SSc platelet releasate[92] potentially due to the anti-angiogenic action of VEGF-A_{165}b. It is not known whether the platelet load of VEGF-A_{xxx}a/$_{xxx}$b isoforms remains consistently elevated in SSc or whether isoform switching occurs at some stage in the disease course.

Additional mediators implicated in enhanced VEGF-A signaling in Systemic sclerosis

Whilst hypoxia is the major driver of VEGF-A expression, other cytokines and growth factors can potentiate VEGF signaling, or are themselves potentiated by VEGF-A expression, which could have important implications for SSc pathogenesis. For example, angiopoietins (Ang-1 and -2) are additional regulators of angiogenesis. Under normoxic conditions, Ang-1 aims to maintain vessel stability through Tie2 signaling, whilst Ang-2 is released under hypoxic stress and acts differentially to either facilitate angiogenesis or angio-regression depending on the presence or absence of VEGF-A respectively[93]. Reported circulating levels of angiopoietins and Tie2 are variable in the literature[84, 88, 93-96]. However, noting the results of a recent study, there is a reduction in Ang-1/-2 ratio in serum of both pre-SSc and SSc with particular association with DU history[93]. Furthermore, increased vascular expression of Ang-2, reduced Tie2 and comparable Ang-1 in SSc skin versus controls[93] potentially represents a shift towards an anti-angiogenic environment. Progressive study regarding the association of Ang-2 with

VEGF-A_{xxx}b isoforms may help map the divergent nature of Ang-2 with VEGF-A expression and the implications of VEGF-A isoforms on angiopoietin function.

Inhibitor of DNA binding protein 1 (Id-1) is a transcription factor and chemokine required for endothelial cell migration and is reduced in SSc endothelial cells, resulting in impaired endothelial cell response to VEGF-A stimulation[97]. The influence of Id-1 expression on responses to specific VEGF-A isoforms has not been investigated.

Increased plasma 8-isoprostane reflects increased oxidative stress in SSc[98] and contributes to impaired angiogenesis[98] via increased TXAR/RhoA/ROCK expression and signaling[98] and subsequent inhibition of VEGF-A induced endothelial cell migration[98]. Interestingly, increased plasma 8-isoprostane appears specific to dcSSc and SSc-ILD and not present in lcSSc and SSc-PAH[98]. Once again, further investigation of these pathways with respect to specific VEGF-A isoforms and correlation with SSc subtype is of interest.

Elevated levels of TGFβ are evident in skin and lung tissue[99] and peripheral B cells[100] in SSc alongside increased TGFβ receptor excretion by cultured SSc fibroblasts[101]. HIF1 increases TGFβ transcription, which in turn stabilizes HIF1α[85, 102](Figure 3). This provides potential for TGFβ mediated indirect VEGF-A stimulation, but it also directly stimulates VEGF-A production in SSc dermal fibroblasts[103]. Furthermore, the effects of HIF and TGFβ on VEGF-A are synergistic in human MVEC in vitro via complimentary action at the HRE on the VEGF promoter region[104]. Moreover, and relevant to SSc pathogenesis, TGFβ encourages a switch from proximal to distal splicing of VEGF-A exon 8 via p38 MAPK signaling[105] favouring VEGF-A_{165}b production in cultured SSc-MVEC[12]. This could ameliorate VEGF-A mediated fibrosis and offer an explanation for the late improvements in skin thickening that characterizes the natural history of SSc. Increased VEGF-A_{165}b in SSc may therefore in part be directed by TGFβ, potentially as part of a negative feedback loop and resulting in mRSS plateau and late improvement.

Treatment of cultured retinal epithelial cells with tumour necrosis factor-α [TNF α], meanwhile, induces a switch from dominant VEGF-A_{165}b at rest to VEGF-A_{165}a[105]. To our knowledge this relationship has not been investigated in SSc specifically. Whilst a previous trial of anti-TNFα agents failed to demonstrate definite improvement in scleroderma[106], the theoretical effects of TNFα inhibition on VEGF-A isoform switching in arresting scleroderma progression is of interest.

PDGF is known to stimulate VEGF-A via phosphatidylinositol 3 kinase[107]. PDGF and its receptors (PDGFR) are increased in SSc and *in vitro*, PDGF can attenuate panVEGF-A production by SSc fibroblasts[11]. Furthermore, there is PDGFR up-regulation (in skin and lung fibroblasts) in response to TGFβ stimulation[108]. Thus, it is possible that PDGF may compliment TGFβ directed VEGF-A activation in SSc fibroblasts.

Hypoxia induces increased synthesis of CTGF mRNA in both healthy and SSc dermal fibroblasts via HIF1α dependent pathways[109]. Circulating CTGF is increased in SSc and associations have been found with diffuse skin disease, pulmonary fibrosis and disease duration[110]. In vitro, CTGF levels are over-expressed in SSc mesenchymal stem cells (MSC) and increased further by VEGF-A stimulation[111].

Caveolins (Cav) are the principle protein constituent of caveolae (cell membrane invaginations that act as 'gate keeper' organelles for a range of cell signaling tasks)[112]. Cav-1 and -2 are the principle caveolins in EC, fibroblasts and adipocytes. Cav-1 acts to down-regulate TGFβ[113, 114] and VEGF-A signaling[111] through receptor internalization providing protection against fibrosis such that Cav-1 knockout mice develop SSc-like features[115]. Accordingly Cav-1 levels and therefore VEGFR2 degradation are reduced in SSc[111, 114] with resultant increase in CTGF expression[111]. Impaired expression of Cav-1 in SSc may therefore contribute to increased VEGF-$A_{xxx}a_{/xxx}$b signaling via VEGFR2.

Cytokines such as interleukin-1β (IL-1β) and Interleukin-6 (IL-6) are pro-inflammatory and pro-fibrotic mediators that induce HIF and VEGF-A through NFκB[53] and signal transducer and activator (Stat3)[116] respectively. Circulating IL-1β is increased in SSc[117] and up-regulates panVEGF-A production *in vitro* SSc fibroblasts[11]. IL-6 is raised in sera[100] and cultured fibroblasts (with further TNFα driven attenuation)[118] and peripheral B cells[100] of patients with SSc, with a correlation between B cell derived IL-6 and mRSS[100]. Accordingly, the potential for anti-IL-6 receptor antibody (Tocilizumab) to treat skin disease in SSc is currently under investigation [119].

Effects of hypoxic design on HIF paralog expression and vascular pathology

In rodent models, differential HIF1α and HIF2α paralog expression occur in chronic intermittent hypoxia ((CIH); as is found in obstructive sleep apnoea) as opposed to chronic continuous hypoxia ((CCH); as occurs in chronic lung disease) and important differences in vascular sequelae occur under these varying hypoxic conditions[30]. Specifically, CIH exposure *induces* HIF1α and *inhibits* HIF2α in mice resulting in systemic hypertension[120, 121] compared to protective effects of heterozygous HIF1α+/- and HIF2α+/- on pulmonary vascular remodeling and PAH in transgenic rodents under CCH[122, 123]. SSc uniquely demonstrates both patterns of intermittent tissue hypoxia with distinct attacks of RP early in disease course, as well as more continuous tissue ischaemia as structural vascular changes progress. We hypothesize, the transition from early disease where vasculopathy and fibrosis are developing to established RP and scleroderma may both be precipitated by and feed forward to influence differential HIF paralog function and downstream VEGF-$A_{xxx}a/_{xxx}b$ signaling. Notionally, this may explain the heterogeneity of vascular manifestations in lcSSc versus dcSSc. Indeed, an association between a HIF1A (gene encoding HIF1α) polymorphism and ACA lcSSc suggests further evaluation of HIF signaling in SSc is warranted [124].

Implications of VEGF-A signaling in the management of Systemic sclerosis

Phosphodiestrase-5 (PDE5) inhibitors and dual endothelin-1 receptor antagonists (ERA) form an integral part of current pharmaceutical therapy for SSc related digital and pulmonary vasculopathy. PDE5 inhibitors effectively improve SSc-RP and digital blood flow through inhibition of cyclic guanosine monophosphate degradation and attenuation of nitric oxide (NO) driven vasodilation[125]. VEGF-A and NO are known reciprocal activators[43], however, PDE5 inhibition in SSc related RP does not appear to alter circulating panVEGF-A levels in sera[125], which may suggest that either NO/VEGF-A potentiation occurs locally in tissues or that by the time PDE5 inhibitors are initiated other factors influencing VEGF-A dominate.

In the previously described in vitro model, Corallo et al., [90] demonstrated the ability of ERA to reduce EndoMT and reverse the ratio of panVEGF-A:VEGF-A_{165}b in favour of angiogenesis. Indeed, in some studies NC patterns demonstrate devolution after ERA therapy[126]. Furthermore, Corrado et al., [127] suggested that ERA may have anti-fibrotic potential in SSc-ILD. Further study to examine the potential of ERA to ameliorate progression of both vasculopathy and fibrosis is warranted.

Drugs directly targeting VEGF signaling are now used in a variety of clinical settings including malignancy[53], retinopathy[53] and IPF[15]. The latter, Nintedanib (a blanket tyrosine kinase inhibitor including VEGFR1-3, PDGFR and fibroblast growth factor receptor,) has been shown to slow disease progression in IPF[15] and is currently being evaluated in SSc-ILD (NCT02597933) following encouraging work using pre-clinical murine models of lung fibrosis, skin fibrosis and PAH[4]. The effect of Nintedanib on VEGF-A_{165}b signaling specifically is however unknown.

Concluding remarks

The evidence presented suggests VEGF-A in particular is an important signaling factor contributing to SSc pathogenesis even at the earliest clinically detectable stages of disease. More precisely, the anti-angiogenic isoform VEGF-A_{165}b contributes to progressive capillary loss and tissue ischaemia. Herein, we have discussed multiple mediators of VEGF signaling and potential implications in SSc, including but not exclusive to: HIF as a major up-regulator of VEGF-A, the divergent angiogenic potential of Ang-1/-2, pro-inflammatory cytokine IL-6 and pro-fibrotic TGFβ with the ability to 'flip the switch' to proximal VEGF-A_{165}b splicing. Of equal importance in the complex SSc story, are the potential cellular sources of VEGF-A isoforms. We have considered with particular interest inflammatory cells, platelets, endothelial cells and fibroblasts; all of which have been demonstrated to produce anti-angiogenic VEGF-

$A_{165}b$ and have been repeatedly implicated in SSc pathology. Whilst SSc is primarily a disease of vascular pathology, fibroblasts undeniably play a role in bolstering the vicious cycle through paracrine action, altering endothelial function and phenotype and encouraging a switch in favour of VEGF-$A_{165}b$ production.

With increasing interest in VEGF-A in SSc, a deeper understanding of the isoform specific responsibilities is required. In particular the role of VEGF-$A_{165}b$ in fibrosis is yet to be elucidated. In collating the literature presented here, we have postulated on our own hypotheses. HIFα paralog expression, determined by the nature of tissue hypoxia and local cytokine expression in SSc may contribute to differential VEGF-A isoform expression and is currently the focus of further investigation. Extrapolating from knowledge in parallel diseases, it may be hypothesized that VEGF-$A_{165}b$ is inhibitory of both angiogenesis and fibrosis and may therefore account for progressive microvascular destruction and the natural regression in skin fibrosis that accompanies established SSc. Furthermore, the relative ratio of VEGF-$A_{xxx}a$:-$A_{xxx}b$ may be important in determining the burden of these clinical features and thus variance in clinical phenotype. This raises the question, whether targeted inhibition of VEGF-$A_{165}b$ may then have beneficial effects on vascular abnormalities but worsen tissue fibrosis? Alternatively, if tissue oxygenation is improved through inhibition of VEGF-$A_{165}b$, would this break the cycle of the Vascular hypothesis and ultimately abrogate both pathologies?
Ultimately, identifying a single molecular target in this multifaceted disease continues to be a challenge. However, VEGF-A and its specific isoforms remain in the spotlight as both potential future biomarkers and therapeutic targets.

Figure legends:

Figure 1 illustrates the evolution of SSc specific nailfold capillaroscopy (NC) changes, from normal through early, active and late patterns[129]. Normal NC pattern as seen in healthy individuals, is recognized by 7-9 regular hairpin shaped capillaries per millimeter. Early pattern maintains capillary number but enlarged* (>20μm limb diameter) and occasionally giant** (>50 μm) capillaries are present. Active pattern shows frequent giant capillaries, microhaemorrhages$^{\infty}$ and some reduction in capillary number. Late pattern is classified primarily by severe capillary loss and evidence of neoangiogenesis$^{\text{⌘}}$ with few/absent giant capillaries/microhaemorrhages.

Figure 2 adapted from collective reports from[43, 45, 48, 53, 130], illustrates vasculogenic actions of VEGF family through their respective signaling receptors including three tyrosine kinase receptors (VEGF receptor-1 (VEGFR1/flt1), VEGF receptor-2 (VEGFR2/KDR/flk1), VEGF receptor-3 (VEGFR3/flt4)), supported by co-receptors (neuropilin-1 (NP-1), neuropilin-2 (NP-2) and heparin sulphate proteoglycan (HSP)). VEGFR2 is the principal receptor for VEGF-A signaling including VEGF-A_{xxx}b isoforms with additional low affinity binding for VEGF-C and VEGF–D following proteolysis. VEGFR1 and VEGFR3 impose regulatory function on VEGFR2. VEGF-A binding to NP-1 and HSP is isoform specific, dependent upon exon splicing[43, 49, 53]. Lack of VEGF-A_{xxx}b affinity for NP-1 contributes to its anti-angiogenic action. Data reporting the affinity of VEGF-A_{121}a for NP1 is mixed and therefore inconclusive[48]. Abbreviations: BM basement membrane, EC endothelial cell, ECM extracellular matrix, HSC haemopoietic stem cells, PlGF placental growth factor, TK tyrosine kinase.

Figure 3 demonstrates the pathway of hypoxia induced VEGF-A induction via HIF and hypoxia mRNA stabilization[32, 43, 53, 104]**.** Known cellular sources of VEGF-A in SSc are illustrated based on available evidence (panVEGF-A = (a), VEGF-A_{165}b = (b))[11, 12, 89, 91, 92, 103, 111, 114, 131]. *TGFβ and HIF1α synergistically increase VEGF-A in endothelial cells[104]. Whilst TGFβ has been shown to favour VEGF-A_{165}b production in SSc-MVEC, similar evidence is not available in other cell lines, where only results for panVEGF-A have been reported. Abbreviations: HRE hypoxia response elements (found in the VEGF-A gene promoter region).

References:

1. Steen VD. Autoantibodies in systemic sclerosis. Seminars in arthritis and rheumatism. 2005;35(1):35-42.
2. Denton CP, Hughes M, Gak N, Vila J, Buch MH, Chakravarty K, et al. BSR and BHPR guideline for the treatment of systemic sclerosis. Rheumatology. 2016;55(10):1906-10.
3. Campbell PM, LeRoy EC. Pathogenesis of systemic sclerosis: a vascular hypothesis. Seminars in arthritis and rheumatism. 1975;4(4):351-68.
4. Distler JH, Feghali-Bostwick C, Soare A, Asano Y, Distler O, Abraham DJ. Review: Frontiers of Antifibrotic Therapy in Systemic Sclerosis. Arthritis & rheumatology. 2017;69(2):257-67.
5. Pope J, McBain D, Petrlich L, Watson S, Vanderhoek L, de Leon F, et al. Imatinib in active diffuse cutaneous systemic sclerosis: Results of a six-month, randomized, double-blind, placebo-controlled, proof-of-concept pilot study at a single center. Arthritis and rheumatism. 2011;63(11):3547-51.
6. Prey S, Ezzedine K, Doussau A, Grandoulier AS, Barcat D, Chatelus E, et al. Imatinib mesylate in scleroderma-associated diffuse skin fibrosis: a phase II multicentre randomized double-blinded controlled trial. The British journal of dermatology. 2012;167(5):1138-44.
7. Denton CP, Merkel PA, Furst DE, Khanna D, Emery P, Hsu VM, et al. Recombinant human anti-transforming growth factor beta1 antibody therapy in systemic sclerosis: a multicenter, randomized, placebo-controlled phase I/II trial of CAT-192. Arthritis and rheumatism. 2007;56(1):323-33.
8. Ioannou M, Pyrpasopoulou A, Simos G, Paraskeva E, Nikolaidou C, Venizelos I, et al. Upregulation of VEGF expression is associated with accumulation of HIF-1 alpha in the skin of naive scleroderma patients. Mod Rheumatol. 2013;23(6):1245-8.
9. Distler O, Del Rosso A, Giacomelli R, Cipriani P, Conforti ML, Guiducci S, et al. Angiogenic and angiostatic factors in systemic sclerosis: increased levels of vascular endothelial growth factor are a feature of the earliest disease stages and are associated with the absence of fingertip ulcers. Arthritis research. 2002;4(6):R11.
10. Choi JJ, Min DJ, Cho ML, Min SY, Kim SJ, Lee SS, et al. Elevated vascular endothelial growth factor in systemic sclerosis. J Rheumatol. 2003;30(7):1529-33.
11. Distler O, Distler JH, Scheid A, Acker T, Hirth A, Rethage J, et al. Uncontrolled expression of vascular endothelial growth factor and its receptors leads to insufficient skin angiogenesis in patients with systemic sclerosis. Circulation research. 2004;95(1):109-16.
12. Manetti M, Guiducci S, Romano E, Ceccarelli C, Bellando-Randone S, Conforti ML, et al. Overexpression of VEGF165b, an inhibitory splice variant of vascular endothelial growth factor, leads to insufficient angiogenesis in patients with systemic sclerosis. Circulation research. 2011;109(3):e14-26.
13. Avouac J, Vallucci M, Smith V, Senet P, Ruiz B, Sulli A, et al. Correlations between angiogenic factors and capillaroscopic patterns in systemic sclerosis. Arthritis research & therapy. 2013;15(2):10.
14. Manetti M, Guiducci S, Romano E, Bellando-Randone S, Lepri G, Bruni C, et al. Increased plasma levels of the VEGF165b splice variant are associated with the severity of nailfold capillary loss in systemic sclerosis. Annals of the rheumatic diseases. 2013;72(8):1425-7.
15. Richeldi L, Cottin V, du Bois RM, Selman M, Kimura T, Bailes Z, et al. Nintedanib in patients with idiopathic pulmonary fibrosis: Combined evidence from the TOMORROW and INPULSIS((R)) trials. Respiratory medicine. 2016;113:74-9.
16. Freemont AJ, Hoyland J, Fielding P, Hodson N, Jayson MI. Studies of the microvascular endothelium in uninvolved skin of patients with systemic sclerosis: direct evidence for a generalized microangiopathy. The British journal of dermatology. 1992;126(6):561-8.
17. Prescott RJ, Freemont AJ, Jones CJ, Hoyland J, Fielding P. Sequential dermal microvascular and perivascular changes in the development of scleroderma. The Journal of pathology. 1992;166(3):255-63.
18. Roumm AD, Whiteside TL, Medsger TA, Jr., Rodnan GP. Lymphocytes in the skin of patients with progressive systemic sclerosis. Quantification, subtyping, and clinical correlations. Arthritis and rheumatism. 1984;27(6):645-53.
19. Meier FM, Frommer KW, Dinser R, Walker UA, Czirjak L, Denton CP, et al. Update on the profile of the EUSTAR cohort: an analysis of the EULAR Scleroderma Trials and Research group database. Annals of the rheumatic diseases. 2012;71(8):1355-60.

20. Koenig M, Joyal F, Fritzler MJ, Roussin A, Abrahamowicz M, Boire G, et al. Autoantibodies and microvascular damage are independent predictive factors for the progression of Raynaud's phenomenon to systemic sclerosis: a twenty-year prospective study of 586 patients, with validation of proposed criteria for early systemic sclerosis. Arthritis and rheumatism. 2008;58(12):3902-12.
21. Sulli A, Ruaro B, Alessandri E, Pizzorni C, Cimmino MA, Zampogna G, et al. Correlations between nailfold microangiopathy severity, finger dermal thickness and fingertip blood perfusion in systemic sclerosis patients. Annals of the rheumatic diseases. 2014;73(1):247-51.
22. De Santis M, Ceribelli A, Cavaciocchi F, Crotti C, Massarotti M, Belloli L, et al. Nailfold videocapillaroscopy and serum VEGF levels in scleroderma are associated with internal organ involvement. Auto- immunity highlights. 2016;7(1):5.
23. Caramaschi P, Canestrini S, Martinelli N, Volpe A, Pieropan S, Ferrari M, et al. Scleroderma patients nailfold videocapillaroscopic patterns are associated with disease subset and disease severity. Rheumatology. 2007;46(10):1566-9.
24. Fichel F, Baudot N, Gaitz JP, Trad S, Barbe C, Frances C, et al. Systemic sclerosis with normal or nonspecific nailfold capillaroscopy. Dermatology. 2014;228(4):360-7.
25. Ingegnoli F, Ardoino I, Boracchi P, Cutolo M, co-authors E. Nailfold capillaroscopy in systemic sclerosis: data from the EULAR scleroderma trials and research (EUSTAR) database. Microvascular research. 2013;89:122-8.
26. Ostojic P, Damjanov N. Different clinical features in patients with limited and diffuse cutaneous systemic sclerosis. Clinical rheumatology. 2006;25(4):453-7.
27. Cutolo M, Herrick AL, Distler O, Becker MO, Beltran E, Carpentier P, et al. Nailfold Videocapillaroscopic Features and Other Clinical Risk Factors for Digital Ulcers in Systemic Sclerosis: A Multicenter, Prospective Cohort Study. Arthritis & rheumatology. 2016;68(10):2527-39.
28. Tolosa-Vilella C, Morera-Morales ML, Simeon-Aznar CP, Mari-Alfonso B, Colunga-Arguelles D, Callejas Rubio JL, et al. Digital ulcers and cutaneous subsets of systemic sclerosis: Clinical, immunological, nailfold capillaroscopy, and survival differences in the Spanish RESCLE Registry. Seminars in arthritis and rheumatism. 2016;46(2):200-8.
29. Sebastiani M, Manfredi A, Colaci M, D'Amico R, Malagoli V, Giuggioli D, et al. Capillaroscopic skin ulcer risk index: a new prognostic tool for digital skin ulcer development in systemic sclerosis patients. Arthritis and rheumatism. 2009;61(5):688-94.
30. Semenza GL. Hypoxia-inducible factors in physiology and medicine. Cell. 2012;148(3):399-408.
31. Card PB, Erbel PJ, Gardner KH. Structural basis of ARNT PAS-B dimerization: use of a common beta-sheet interface for hetero- and homodimerization. Journal of molecular biology. 2005;353(3):664-77.
32. Deng W, Feng X, Li X, Wang D, Sun L. Hypoxia-inducible factor 1 in autoimmune diseases. Cellular immunology. 2016;303:7-15.
33. Doedens A, Johnson RS. Transgenic Models to Understand Hypoxia‐Inducible Factor Function. Methods in Enzymology. Oxygen Biology and Hypoxia. Volume 435: Academic Press; 2007. p. 87-105.
34. Zhou G, Dada LA, Wu M, Kelly A, Trejo H, Zhou Q, et al. Hypoxia-induced alveolar epithelial-mesenchymal transition requires mitochondrial ROS and hypoxia-inducible factor 1. American journal of physiology Lung cellular and molecular physiology. 2009;297(6):L1120-30.
35. Lei W, He Y, Shui X, Li G, Yan G, Zhang Y, et al. Expression and analyses of the HIF-1 pathway in the lungs of humans with pulmonary arterial hypertension. Molecular medicine reports. 2016;14(5):4383-90.
36. Senger DR, Galli SJ, Dvorak AM, Perruzzi CA, Harvey VS, Dvorak HF. Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Science. 1983;219(4587):983-5.
37. Ferrara N, Henzel WJ. Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. Biochemical and biophysical research communications. 1989;425(3):540-7.
38. Leung DW, Cachianes G, Kuang WJ, Goeddel DV, Ferrara N. Vascular endothelial growth factor is a secreted angiogenic mitogen. Science. 1989;246(4935):1306-9.
39. Carmeliet P, Ferreira V, Breier G, Pollefeyt S, Kieckens L, Gertsenstein M, et al. Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. Nature. 1996;380(6573):435-9.
40. Fong GH, Rossant J, Gertsenstein M, Breitman ML. Role of the Flt-1 receptor tyrosine kinase in regulating the assembly of vascular endothelium. Nature. 1995;376(6535):66-70.

41. Hu K, Olsen BR. The roles of vascular endothelial growth factor in bone repair and regeneration. Bone. 2016;91:30-8.
42. Barratt SL, Blythe T, Jarrett C, Ourradi K, Shelley-Fraser G, Day MJ, et al. Differential Expression of VEGF-Axxx Isoforms Is Critical for Development of Pulmonary Fibrosis. American journal of respiratory and critical care medicine. 2017;196(4):479-93.
43. Neufeld G, Cohen T, Gengrinovitch S, Poltorak Z. Vascular endothelial growth factor (VEGF) and its receptors. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 1999;13(1):9-22.
44. Byrne AM, Bouchier-Hayes DJ, Harmey JH. Angiogenic and cell survival functions of vascular endothelial growth factor (VEGF). Journal of cellular and molecular medicine. 2005;9(4):777-94.
45. Alvarez-Aznar A, Muhl L, Gaengel K. VEGF Receptor Tyrosine Kinases: Key Regulators of Vascular Function. Current topics in developmental biology. 2017;123:433-82.
46. Hoeben A, Landuyt B, Highley MS, Wildiers H, Van Oosterom AT, De Bruijn EA. Vascular endothelial growth factor and angiogenesis. Pharmacol Rev. 2004;56(4):549-80.
47. Bates DO, Cui TG, Doughty JM, Winkler M, Sugiono M, Shields JD, et al. VEGF165b, an inhibitory splice variant of vascular endothelial growth factor, is down-regulated in renal cell carcinoma. Cancer research. 2002;62(14):4123-31.
48. Delcombel R, Janssen L, Vassy R, Gammons M, Haddad O, Richard B, et al. New prospects in the roles of the C-terminal domains of VEGF-A and their cooperation for ligand binding, cellular signaling and vessels formation. Angiogenesis. 2013;16(2):353-71.
49. Eswarappa SM, Fox PL. Antiangiogenic VEGF-Ax: A New Participant in Tumor Angiogenesis. Cancer research. 2015;75(14):2765-9.
50. Manetti M, Guiducci S, Matucci-Cerinic M. The crowded crossroad to angiogenesis in systemic sclerosis: where is the key to the problem? Arthritis research & therapy. 2016;18:36.
51. Ballmer-Hofer K, Andersson AE, Ratcliffe LE, Berger P. Neuropilin-1 promotes VEGFR-2 trafficking through Rab11 vesicles thereby specifying signal output. Blood. 2011;118(3):816-26.
52. Bills VL, Varet J, Millar A, Harper SJ, Soothill PW, Bates DO. Failure to up-regulate VEGF165b in maternal plasma is a first trimester predictive marker for pre-eclampsia. Clinical science. 2009;116(3):265-72.
53. Ramakrishnan S, Anand V, Roy S. Vascular endothelial growth factor signaling in hypoxia and inflammation. J Neuroimmune Pharmacol. 2014;9(2):142-60.
54. Avouac J, Wipff J, Goldman O, Ruiz B, Couraud PO, Chiocchia G, et al. Angiogenesis in Systemic Sclerosis Impaired Expression of Vascular Endothelial Growth Factor Receptor 1 in Endothelial Progenitor-Derived Cells Under Hypoxic Conditions. Arthritis and rheumatism. 2008;58(11):3550-61.
55. Silva I, Teixeira A, Oliveira J, Almeida I, Almeida R, Aguas A, et al. Endothelial Dysfunction and Nailfold Videocapillaroscopy Pattern as Predictors of Digital Ulcers in Systemic Sclerosis: a Cohort Study and Review of the Literature. Clinical reviews in allergy & immunology. 2015;49(2):240-52.
56. McMahan Z, Schoenhoff F, Van Eyk JE, Wigley FM, Hummers LK. Biomarkers of pulmonary hypertension in patients with scleroderma: a case-control study. Arthritis research & therapy. 2015;17:201.
57. Glodkowska-Mrowka E, Gorska E, Ciurzynski M, Stelmaszczyk-Emmel A, Bienias P, Irzyk K, et al. Pro- and antiangiogenic markers in patients with pulmonary complications of systemic scleroderma. Respir Physiol Neurobiol. 2015;209:69-75.
58. Gigante A, Navarini L, Margiotta D, Amoroso A, Barbano B, Cianci R, et al. Angiogenic and angiostatic factors in renal scleroderma-associated vasculopathy. Microvascular research. 2017;114:41-5.
59. Rentka A, Harsfalvi J, Berta A, Koroskenyi K, Szekanecz Z, Szucs G, et al. Vascular Endothelial Growth Factor in Tear Samples of Patients with Systemic Sclerosis. Mediators of inflammation. 2015;2015:573681.
60. Choi JJ, Min DJ, Cho ML, Min SY, Kim SJ, Lee SS, et al. Elevated vascular endothelial growth factor in systemic sclerosis. The Journal of rheumatology. 2003;30(7):1529-33.
61. Avouac J, Vallucci M, Smith V, Senet P, Ruiz B, Sulli A, et al. Correlations between angiogenic factors and capillaroscopic patterns in systemic sclerosis. Arthritis research & therapy. 2013;15(2):R55.
62. Kaner RJ, Crystal RG. Compartmentalization of vascular endothelial growth factor to the epithelial surface of the human lung. Molecular medicine (Cambridge, Mass). 2001;7(4):240-6.

63. Avouac J, Wipff J, Goldman O, Ruiz B, Couraud PO, Chiocchia G, et al. Angiogenesis in systemic sclerosis: impaired expression of vascular endothelial growth factor receptor 1 in endothelial progenitor-derived cells under hypoxic conditions. Arthritis and rheumatism. 2008;58(11):3550-61.
64. Jinnin M, Makino T, Kajihara I, Honda N, Makino K, Ogata A, et al. Serum levels of soluble vascular endothelial growth factor receptor-2 in patients with systemic sclerosis. The British journal of dermatology. 2010;162(4):751-8.
65. Romano E, Chora I, Manetti M, Mazzotta C, Rosa I, Bellando-Randone S, et al. Decreased expression of neuropilin-1 as a novel key factor contributing to peripheral microvasculopathy and defective angiogenesis in systemic sclerosis. Annals of the rheumatic diseases. 2016;75(8):1541-9.
66. Higashi-Kuwata N, Makino T, Inoue Y, Ihn H. Expression pattern of VEGFR-1,-2,-3 and D2-40 protein in the skin of patients with systemic sclerosis. Eur J Dermatol. 2011;21(4):490-4.
67. Chora I, Romano E, Manetti M, Mazzotta C, Costa R, Machado V, et al. Evidence for a Derangement of the Microvascular System in Patients with a Very Early Diagnosis of Systemic Sclerosis. The Journal of rheumatology. 2017;44(8):1190-7.
68. Smith V, Decuman S, Sulli A, Bonroy C, Piettte Y, Deschepper E, et al. Do worsening scleroderma capillaroscopic patterns predict future severe organ involvement? a pilot study. Annals of the rheumatic diseases. 2012;71(10):1636-9.
69. Maurer B, Distler A, Suliman YA, Gay RE, Michel BA, Gay S, et al. Vascular endothelial growth factor aggravates fibrosis and vasculopathy in experimental models of systemic sclerosis. Annals of the rheumatic diseases. 2014;73(10):1880-7.
70. Dor Y, Djonov V, Abramovitch R, Itin A, Fishman GI, Carmeliet P, et al. Conditional switching of VEGF provides new insights into adult neovascularization and pro-angiogenic therapy. The EMBO journal. 2002;21(8):1939-47.
71. Manetti M, Milia AF, Guiducci S, Romano E, Matucci-Cerinic M, Ibba-Manneschi L. Progressive Loss of Lymphatic Vessels in Skin of Patients with Systemic Sclerosis. J Rheumatol. 2011;38(2):297-301.
72. Honda N, Jinnin M, Kajihara I, Makino T, Fukushima S, Ihn H. Impaired lymphangiogenesis due to excess vascular endothelial growth factor-D/Flt-4 signalling in the skin of patients with systemic sclerosis. Br J Dermatol. 2010;163(4):776-80.
73. Chitale S, Al-Mowallad AF, Wang Q, Kumar S, Herrick A. High circulating levels of VEGF-C suggest abnormal lymphangiogenesis in systemic sclerosis. Rheumatology. 2008;47(11):1727-8.
74. Kylhammar D, Hesselstrand R, Nielsen S, Scheele C, Radegran G. Angiogenic and inflammatory biomarkers for screening and follow-up in patients with pulmonary arterial hypertension. Scandinavian journal of rheumatology. 2018:1-6.
75. Porkholm M, Bono P, Saarinen-Pihkala UM, Kivivuori SM. Higher angiopoietin-2 and VEGF levels predict shorter EFS and increased non-relapse mortality after pediatric hematopoietic SCT. Bone marrow transplantation. 2013;48(1):50-5.
76. Min CK, Kim SY, Lee MJ, Eom KS, Kim YJ, Kim HJ, et al. Vascular endothelial growth factor (VEGF) is associated with reduced severity of acute graft-versus-host disease and nonrelapse mortality after allogeneic stem cell transplantation. Bone marrow transplantation. 2006;38(2):149-56.
77. Kim DH, Lee NY, Lee MH, Sohn SK. Vascular endothelial growth factor gene polymorphisms may predict the risk of acute graft-versus-host disease following allogeneic transplantation: preventive effect of vascular endothelial growth factor gene on acute graft-versus-host disease. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation. 2008;14(12):1408-16.
78. Holtan SG, Verneris MR, Schultz KR, Newell LF, Meyers G, He F, et al. Circulating Angiogenic Factors Associated with Response and Survival in Patients with Acute Graft-versus-Host Disease: Results from Blood and Marrow Transplant Clinical Trials Network 0302 and 0802. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation. 2015;21(6):1029-36.
79. Yao Y, Wang L, Zhou J, Zhang X. HIF-1alpha inhibitor echinomycin reduces acute graft-versus-host disease and preserves graft-versus-leukemia effect. Journal of translational medicine. 2017;15(1):28.
80. Wang J, Lu Z, Xu Z, Tian P, Miao H, Pan S, et al. Reduction of hepatic fibrosis by overexpression of von Hippel-Lindau protein in experimental models of chronic liver disease. Sci Rep. 2017;7:41038.
81. Tzouvelekis A, Harokopos V, Paparountas T, Oikonomou N, Chatziioannou A, Vilaras G, et al. Comparative expression profiling in pulmonary fibrosis suggests a role of hypoxia-inducible factor-1alpha in disease pathogenesis. American journal of respiratory and critical care medicine. 2007;176(11):1108-19.

82. Zhao ZM, Liu HL, Sun X, Guo T, Shen L, Tao YY, et al. Levistilide A inhibits angiogenesis in liver fibrosis via vascular endothelial growth factor signaling pathway. Experimental biology and medicine (Maywood, NJ). 2017;242(9):974-85.
83. Maurer B, Distler A, Suliman YA, Gay RE, Michel BA, Gay S, et al. Vascular endothelial growth factor aggravates fibrosis and vasculopathy in experimental models of systemic sclerosis. Annals of the rheumatic diseases. 2013.
84. Riccieri V, Stefanantoni K, Vasile M, Macri V, Sciarra I, Iannace N, et al. Abnormal plasma levels of different angiogenic molecules are associated with different clinical manifestations in patients with systemic sclerosis. Clinical and experimental rheumatology. 2011;29(2 Suppl 65):S46-52.
85. Distler JH, Jungel A, Pileckyte M, Zwerina J, Michel BA, Gay RE, et al. Hypoxia-induced increase in the production of extracellular matrix proteins in systemic sclerosis. Arthritis and rheumatism. 2007;56(12):4203-15.
86. Lauer BM, Baechler, E.C., Molitor, J.A., editor The anti-angiogenic VEGF-165b isoform is elevated in both anti-centromere and anti-topoisomerase positive systemic sclerosis patients. 13th International Workshop on Scleroderma Research; 2013 3rd August 2013; Boston University, Boston, Massachusetts.
87. Riccieri V, Stefanantoni K, Vasile M, Macri V, Sciarra I, Iannace N, et al. Abnormal plasma levels of different angiogenic molecules are associated with different clinical manifestations in patients with systemic sclerosis. Clinical and experimental rheumatology. 2011;29(2):S46-S52.
88. Cossu M, Andracco R, Santaniello A, Marchini M, Severino A, Caronni M, et al. Serum levels of vascular dysfunction markers reflect disease severity and stage in systemic sclerosis patients. Rheumatology. 2016;55(6):1112-6.
89. Bielecki M, Kowal K, Lapinska A, Chwiesko-Minarowska S, Chyczewski L, Kowal-Bielecka O. Peripheral blood mononuclear cells from patients with systemic sclerosis spontaneously secrete increased amounts of vascular endothelial growth factor (VEGF) already in the early stage of the disease. Advances in Medical Sciences. 2011;56(2):255-63.
90. Corallo C, Cutolo M, Kahaleh B, Pecetti G, Montella A, Chirico C, et al. Bosentan and macitentan prevent the endothelial-to-mesenchymal transition (EndoMT) in systemic sclerosis: in vitro study. Arthritis research & therapy. 2016;18(1):228.
91. Solanilla A, Villeneuve J, Auguste P, Hugues M, Alioum A, Lepreux S, et al. The transport of high amounts of vascular endothelial growth factor by blood platelets underlines their potential contribution in systemic sclerosis angiogenesis. Rheumatology. 2009;48(9):1036-44.
92. Hirigoyen D, Burgos PI, Mezzano V, Duran J, Barrientos M, Saez CG, et al. Inhibition of angiogenesis by platelets in systemic sclerosis patients. Arthritis research & therapy. 2015;17:332.
93. Moritz F, Schniering J, Distler JHW, Gay RE, Gay S, Distler O, et al. Tie2 as a novel key factor of microangiopathy in systemic sclerosis. Arthritis research & therapy. 2017;19(1):105.
94. Michalska-Jakubus M, Kowal-Bielecka O, Chodorowska G, Bielecki M, Krasowska D. Angiopoietins-1 and -2 are differentially expressed in the sera of patients with systemic sclerosis: high angiopoietin-2 levels are associated with greater severity and higher activity of the disease. Rheumatology. 2011;50(4):746-55.
95. Noda S, Asano Y, Aozasa N, Akamata K, Yamada D, Masui Y, et al. Serum Tie2 levels: clinical association with microangiopathies in patients with systemic sclerosis. Journal of the European Academy of Dermatology and Venereology : JEADV. 2011;25(12):1476-9.
96. Dunne JV, Keen KJ, Van Eeden SF. Circulating angiopoietin and Tie-2 levels in systemic sclerosis. Rheumatology international. 2013;33(2):475-84.
97. Tsou PS, Rabquer BJ, Ohara RA, Stinson WA, Campbell PL, Amin MA, et al. Scleroderma dermal microvascular endothelial cells exhibit defective response to pro-angiogenic chemokines. Rheumatology. 2016;55(4):745-54.
98. Tsou PS, Amin MA, Campbell PL, Zakhem G, Balogh B, Edhayan G, et al. Activation of the Thromboxane A2 Receptor by 8-Isoprostane Inhibits the Pro-Angiogenic Effect of Vascular Endothelial Growth Factor in Scleroderma. J Invest Dermatol. 2015;135(12):3153-62.
99. Denton CP, Abraham DJ. Transforming growth factor-beta and connective tissue growth factor: key cytokines in scleroderma pathogenesis. Current opinion in rheumatology. 2001;13(6):505-11.
100. Dumoitier N, Chaigne B, Regent A, Lofek S, Mhibik M, Dorfmuller P, et al. Scleroderma peripheral B lymphocytes secrete interleukin-6 and TGF-beta and activate fibroblasts. Arthritis & rheumatology. 2016.
101. Yamane K, Ihn H, Kubo M, Tamaki K. Increased transcriptional activities of transforming growth factor beta receptors in scleroderma fibroblasts. Arthritis and rheumatism. 2002;46(9):2421-8.

102. McMahon S, Charbonneau M, Grandmont S, Richard DE, Dubois CM. Transforming growth factor beta1 induces hypoxia-inducible factor-1 stabilization through selective inhibition of PHD2 expression. The Journal of biological chemistry. 2006;281(34):24171-81.
103. Kajihara I, Jinnin M, Honda N, Makino K, Makino T, Masuguchi S, et al. Scleroderma dermal fibroblasts overexpress vascular endothelial growth factor due to autocrine transforming growth factor beta signaling. Modern rheumatology / the Japan Rheumatism Association. 2013;23(3):516-24.
104. Sanchez-Elsner T, Botella LM, Velasco B, Corbi A, Attisano L, Bernabeu C. Synergistic cooperation between hypoxia and transforming growth factor-beta pathways on human vascular endothelial growth factor gene expression. The Journal of biological chemistry. 2001;276(42):38527-35.
105. Nowak DG, Woolard J, Amin EM, Konopatskaya O, Saleem MA, Churchill AJ, et al. Expression of pro- and anti-angiogenic isoforms of VEGF is differentially regulated by splicing and growth factors. Journal of cell science. 2008;121(Pt 20):3487-95.
106. Denton CP, Engelhart M, Tvede N, Wilson H, Khan K, Shiwen X, et al. An open-label pilot study of infliximab therapy in diffuse cutaneous systemic sclerosis. Annals of the rheumatic diseases. 2009;68(9):1433-9.
107. Wang D, Huang HJ, Kazlauskas A, Cavenee WK. Induction of vascular endothelial growth factor expression in endothelial cells by platelet-derived growth factor through the activation of phosphatidylinositol 3-kinase. Cancer research. 1999;59(7):1464-72.
108. Trojanowska M. Role of PDGF in fibrotic diseases and systemic sclerosis. Rheumatology. 2008;47 Suppl 5:v2-4.
109. Hong KH, Yoo SA, Kang SS, Choi JJ, Kim WU, Cho CS. Hypoxia induces expression of connective tissue growth factor in scleroderma skin fibroblasts. Clinical and experimental immunology. 2006;146(2):362-70.
110. Sato S, Nagaoka T, Hasegawa M, Tamatani T, Nakanishi T, Takigawa M, et al. Serum levels of connective tissue growth factor are elevated in patients with systemic sclerosis: association with extent of skin sclerosis and severity of pulmonary fibrosis. The Journal of rheumatology. 2000;27(1):149-54.
111. Cipriani P, Di Benedetto P, Capece D, Zazzeroni F, Liakouli V, Ruscitti P, et al. Impaired Cav-1 expression in SSc mesenchymal cells upregulates VEGF signaling: a link between vascular involvement and fibrosis. Fibrogenesis & tissue repair. 2014;7:13.
112. Jasmin J-Fo. Caveolins and Caveolae Roles in Signaling and Disease Mechanisms. Frank PG, Lisanti MP, editors. New York, NY: New York, NY : Springer US; 2012.
113. Di Guglielmo GM, Le Roy C, Goodfellow AF, Wrana JL. Distinct endocytic pathways regulate TGF-beta receptor signalling and turnover. Nature cell biology. 2003;5(5):410-21.
114. Del Galdo F, Sotgia F, de Almeida CJ, Jasmin JF, Musick M, Lisanti MP, et al. Decreased expression of caveolin 1 in patients with systemic sclerosis: crucial role in the pathogenesis of tissue fibrosis. Arthritis and rheumatism. 2008;58(9):2854-65.
115. Castello-Cros R, Whitaker-Menezes D, Molchansky A, Purkins G, Soslowsky LJ, Beason DP, et al. Scleroderma-like properties of skin from caveolin-1-deficient mice: implications for new treatment strategies in patients with fibrosis and systemic sclerosis. Cell cycle. 2011;10(13):2140-50.
116. Xu Q, Briggs J, Park S, Niu G, Kortylewski M, Zhang S, et al. Targeting Stat3 blocks both HIF-1 and VEGF expression induced by multiple oncogenic growth signaling pathways. Oncogene. 2005;24(36):5552-60.
117. Zhang YJ, Zhang Q, Yang GJ, Tao JH, Wu GC, Huang XL, et al. Elevated serum levels of interleukin-1beta and interleukin-33 in patients with systemic sclerosis in Chinese population. Zeitschrift fur Rheumatologie. 2016.
118. Antonelli A, Fallahi P, Ferrari SM, Giuggioli D, Colaci M, Di Domenicantonio A, et al. Systemic sclerosis fibroblasts show specific alterations of interferon-gamma and tumor necrosis factor-alpha-induced modulation of interleukin 6 and chemokine ligand 2. The Journal of rheumatology. 2012;39(5):979-85.
119. Khanna D, Denton CP, Jahreis A, van Laar JM, Frech TM, Anderson ME, et al. Safety and efficacy of subcutaneous tocilizumab in adults with systemic sclerosis (faSScinate): a phase 2, randomised, controlled trial. Lancet. 2016;387(10038):2630-40.
120. Peng YJ, Yuan G, Ramakrishnan D, Sharma SD, Bosch-Marce M, Kumar GK, et al. Heterozygous HIF-1alpha deficiency impairs carotid body-mediated systemic responses and reactive oxygen species generation in mice exposed to intermittent hypoxia. The Journal of physiology. 2006;577(Pt 2):705-16.

121. Peng YJ, Yuan G, Khan S, Nanduri J, Makarenko VV, Reddy VD, et al. Regulation of hypoxia-inducible factor-alpha isoforms and redox state by carotid body neural activity in rats. The Journal of physiology. 2014;592(17):3841-58.
122. Li Y, Shi B, Huang L, Wang X, Yu X, Guo B, et al. Suppression of the expression of hypoxia-inducible factor-1alpha by RNA interference alleviates hypoxia-induced pulmonary hypertension in adult rats. International journal of molecular medicine. 2016;38(6):1786-94.
123. Brusselmans K, Compernolle V, Tjwa M, Wiesener MS, Maxwell PH, Collen D, et al. Heterozygous deficiency of hypoxia-inducible factor-2alpha protects mice against pulmonary hypertension and right ventricular dysfunction during prolonged hypoxia. The Journal of clinical investigation. 2003;111(10):1519-27.
124. Wipff J, Dieude P, Avouac J, Tiev K, Hachulla E, Granel B, et al. Association of hypoxia-inducible factor 1A (HIF1A) gene polymorphisms with systemic sclerosis in a French European Caucasian population. Scandinavian journal of rheumatology. 2009;38(4):291-4.
125. Andrigueti FV, Ebbing PCC, Arismendi MI, Kayser C. Evaluation of the effect of sildenafil on the microvascular blood flow in patients with systemic sclerosis: a randomised, double-blind, placebo-controlled study. Clinical and experimental rheumatology. 2017;35 Suppl 106(4):151-8.
126. Guiducci S, Bellando Randone S, Bruni C, Carnesecchi G, Maresta A, Iannone F, et al. Bosentan fosters microvascular de-remodelling in systemic sclerosis. Clinical rheumatology. 2012;31(12):1723-5.
127. Corrado A, Neve A, Costantino E, Palladino GP, Foschino Barbaro MP, Cantatore FP. Effect of endothelin inhibition on lung fibroblasts on patients with systemic sclerosis. Minerva medica. 2013;104(5):505-17.
128. Gammons MV, Dick AD, Harper SJ, Bates DO. SRPK1 inhibition modulates VEGF splicing to reduce pathological neovascularization in a rat model of retinopathy of prematurity. Investigative ophthalmology & visual science. 2013;54(8):5797-806.
129. Cutolo M, Pizzorni C, Tuccio M, Burroni A, Craviotto C, Basso M, et al. Nailfold videocapillaroscopic patterns and serum autoantibodies in systemic sclerosis. Rheumatology. 2004;43(6):719-26.
130. Heinolainen K, Karaman S, D'Amico G, Tammela T, Sormunen R, Eklund L, et al. VEGFR3 Modulates Vascular Permeability by Controlling VEGF/VEGFR2 Signaling. Circulation research. 2017.
131. Ioannou M, Pyrpasopoulou A, Simos G, Paraskeva E, Nikolaidou C, Venizelos I, et al. Upregulation of VEGF expression is associated with accumulation of HIF-1alpha in the skin of naive scleroderma patients. Modern rheumatology / the Japan Rheumatism Association. 2013;23(6):1245-8.